Detailed Assessment of Renal Function in a Proband with Harboyan Syndrome Caused by a Novel Homozygous *SLC4A11* Nonsense Mutation

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Key Words

Harboyan syndrome · *SLC4A11* · Corneal dystrophy · Sensorineural hearing loss · Kidney function tests

Abstract

Background/Aims: To identify the underlying molecular genetic cause of disease in a patient with Harboyan syndrome and to perform a detailed assessment of her renal function. We also assessed the influence of the *SLC4A11* mutation identified on the corneal endothelium in the heterozygous state. **Methods:** A 55-year-old female was examined ophthalmologically, audiotically and nephrologically including 24-hour urine collection. The coding region of *SLC4A11* was directly sequenced. Specular microscopy was performed in the proband's 21-year-old daughter. **Results:** The proband had bilateral iridectomy at the age of 3 months because of an initial diagnosis of congenital glaucoma and since the age of 12 years she underwent several keratoplasties in each eye. Nephrological examination did not reveal any abnormalities. Moderate bilateral sensorineural hearing loss was confirmed by audiometry. A novel homozygous mutation predicted to lead to a premature stop codon at the protein level, c.2188C>T; p.(Arg730*), was identified in *SLC4A11*. No changes in corneal endothelial cell morphology

or density were observed in the heterozygous daughter. **Conclusion:** In contrast to the *Slc4a11*^{-/-} mouse, no abnormalities in daily renal ion excretion or polyuria were observed in the Harboyan syndrome patient. The mutation identified does not affect corneal endothelial cell morphology or density in the heterozygous state.

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Introduction

Harboyan syndrome (OMIM No. 217400) is a rare recessive disorder characterized by the presence of congenital hereditary endothelial dystrophy (CHED) manifesting as congenital corneal edema in combination with the development of sensorineural hearing impairment usually in the second decade of life [1]. The disorder is caused by mutations in *SLC4A11* that encodes sodium bicarbonate transporter-like protein 11 (SLC4A11). Mutations within *SLC4A11* are also responsible for the development of isolated recessive CHED (CHED2, OMIM No. 217700) [1–3]. In addition, heterozygous missense *SLC4A11* mutations have been identified in a subset of patients with dominantly inherited late-onset Fuchs' endothelial corneal dystrophy (FECD4, OMIM No. 613268) [4, 5].

In humans *SLC4A11* is predominantly expressed in the kidney, salivary glands, thyroid gland, testis and trachea [6]. *SLC4A11* is a plasma membrane protein with Na⁺-dependent OH⁻ co-transport activity contributing to intracellular pH regulation [7] and with a direct role in transmembrane water flux in response to osmotic gradients [8]. Loss of functional *SLC4A11* also induces apoptosis in human immortalized corneal endothelial cells [9]. Mouse studies have focused on the characterization of *Slc4a11* function in the cornea, kidney and inner ear [10–12]. *Slc4a11*^{-/-} mice exhibit defects in fluid resorption in the kidney in addition to a CHED phenotype and sensorineural deafness [10–12]. In this study we describe a Czech proband with Harboyan syndrome caused by a presumably pathogenic novel homozygous *SLC4A11* nonsense variant, with detailed clinical findings including analysis of renal function.

Methods

Clinical Evaluation

Ophthalmological assessment included best-corrected visual acuity measured on Snellen charts, slit-lamp biomicroscopy and intraocular pressure measurements.

One of the two proband's children was examined by means of specular microscopy (Noncon ROBO Pachy SP-9000, Konan Medical Inc., Tokyo, Japan). Endothelial cell density was measured for each eye at three different locations and measurements obtained were averaged.

Three consecutive 24-hour urine collections were performed. Samples were examined for the following urinary parameters: osmolality, total volume, creatinine, sodium, potassium, calcium, inorganic phosphate, chloride, protein, creatinine clearance, uric acid and urea nitrogen. Normal values for 24-hour urine collection were based on sex-specific ranges and were consistent with established definitions [13]. Polyuria was defined as diuresis >3 litres/day [13].

Audiometry was performed bilaterally. Mild hearing impairment was defined as loss of 21–40 dB, moderate as 41–60 dB, severe as 61–90 dB and profound as >90 dB loss.

Other complementary investigations undertaken comprised laboratory blood tests for metabolism (sodium, potassium, chloride, magnesium, calcium, phosphorus, bicarbonate, blood urea nitrogen, uric acid, creatinine, fasting glucose), blood cell count, blood gas analysis, liver function tests, lipid panel, albumin, C-reactive protein, protein electrophoresis, thyroid hormone tests, immunoglobulins (IgA, IgG, IgM, IgE), complement component 3 and 4, rheumatoid factor, serum protein electrophoresis, nitrogen metabolites, echocardiography, chest X-ray, abdominal ultrasonography, and diffusing capacity of lungs for carbon monoxide.

Molecular Genetic Analysis

The proband's DNA was isolated from whole blood using the Gentra Puregene™ Blood Kit (Qiagen, Hilden, Germany). DNA from her clinically unaffected daughter was extracted using an Oragene saliva collection kit (DNA Genotek, Inc., Kanata, Canada).

PCR amplification and Sanger sequencing of 19 *SLC4A11* coding exons was performed using previously published primers [3]. Conditions of PCR and sequencing reactions are available upon request.

All 19 protein coding exons of *SLC4A11* were screened in the proband, whereas her unaffected daughter was only analysed for exon 16. The derived sequences were aligned to NM_032034.3 using the DNASTAR package software version 10.1.2 (Lasergene, Madison, Wisc., USA). The 1000 Genomes data set (<http://browser.1000genomes.org/>) and the Exome Variant Server (EVS, <http://evs.gs.washington.edu/EVS/>) were assessed on January 16 to determine the frequency of *SLC4A11* variants. Mutation description followed the recommendations of the Human Genome Variation Society (www.hgvs.org/mutnomen/) [14].

The research was conducted in accordance with the Declaration of Helsinki and institutional ethics committee approval was obtained prior to the start of the study. Investigated subjects have signed informed consent.

Results

The proband, a 55-year-old Caucasian Czech female, reported no family history of consanguinity, corneal disease or deafness. She was an only child, and both of her deceased parents originated in a small town with a population of approximately 8,500 inhabitants.

Ocular history indicated the presence of cloudy corneas at birth. At that time she was diagnosed with congenital glaucoma and underwent bilateral iridectomy at the age of 3 months.

She was first examined in the Department of Ophthalmology in Prague at the age of 31 years. Bilateral mixed horizontal-rotatory nystagmus, failed graft in the right eye and clear graft in the left eye were noted. Corneal diameters were bilaterally less than 12 mm. During the course of her life three penetrating keratoplasties were performed in the right eye and one lamellar keratoplasty, one penetrating keratoplasty and one Descemet stripping automated endothelial keratoplasty in the left eye (online suppl. table 1, www.karger.com/doi/10.1159/000365109). At the age of 55 years her best-corrected visual acuity was 0.05 in the right eye and 0.01 in the left eye with pin hole (1.5 months after Descemet stripping automated endothelial keratoplasty). Since 31 years of age she had been using topical antiglaucomatous medication in both eyes. During the follow-up for which medical records were available, several increased intraocular pressure spikes were detected. Visual field examinations did not support signs of glaucomatous optic disc cupping. Myopic degeneration was detected bilaterally on fundus examination, however, no detailed assessment could be performed due to the anterior segment findings. Because of the congenital onset, a differential diagnosis included CHED2 and

posterior polymorphous corneal dystrophy, that occasionally also exhibits early onset [15].

The patient had audiological testing in the second decade of life because of a suspicion of hearing impairment; however, results of this examination were not available. She noticed hearing impairment that precluded daily tasks in her fourth decade of life. Audiometry performed at the age of 51 years confirmed moderate bilateral symmetrical perception deafness that suggested a likely diagnosis of Harboyan syndrome.

The total volume of the 24-hour urine collection was 2,500 ml for each sample (with a normal fluid intake of about 2 litres/day). Normal levels of all examined parameters were detected (table 1). Results of other complementary investigations were all within the normal range.

Genetic analysis of the 19 *SLC4A11* coding exons revealed a novel homozygous mutation c.2188C>T in exon 16 (fig. 1a) leading to the introduction of a premature termination codon at position 730 [p.(Arg730*)]. The sequence change was not found in the 1000 Genomes data set or EVS. No other sequence variants were identified within the coding region or splice sites of *SLC4A11*.

Specular microscopy examination in the 21-year-old daughter of the proband, who was a heterozygous carrier for the c.2188C>T mutation (fig. 1b), showed normal endothelial cell morphology with an averaged endothelial cell density of 2,892 cells/mm² in the right eye (fig. 2a) and 3,077 cells/mm² in the left eye (fig. 2b) as compared to an age-matched control individual (fig. 2c). Normal endothelial cell density values of 2,940 (±345) cells/mm² were defined using the same type of specular microscope in a study by Galgauskas et al. [16] and refer to findings in healthy right eyes from 30 young individuals (mean age ± SD: 23.5 ± 0.1 years). Central corneal thickness was 563 µm in the right eye and 561 µm in the left eye (normal range ± SD: 555 ± 43 µm) [16].

Discussion

Mutations within *SLC4A11* have been associated in humans with the development of both recessive and dominant corneal endothelial dystrophies [3–5, 17] as well as recessive Harboyan syndrome presenting as congenital hereditary endothelial dystrophy with sensorineural deafness [2].

In this study, in a proband with Harboyan syndrome we have detected a novel, likely pathogenic homozygous nonsense variant in *SLC4A11*, c.2188C>T; [p.(Arg730*)]. If the transcript is not eliminated by nonsense-mediated

Fig. 1. Sequence chromatograms. Homozygous *SLC4A11* C>T transition at nucleotide c.2188 in a Czech proband with Harboyan syndrome (a). Heterozygous change in the proband's daughter (b). RefSeq shows the NCBI Reference Sequence NM_032034.3.

Table 1. Measurements from 24-hour urinary collection in a proband with Harboyan syndrome

Parameter	Proband	Normal range
Urine osmolality, mmol/kg	334	50–854
pH	5.5	5–7
Creatinine, mmol/day	8.8	8.8–13.3
Urea nitrogen, mmol/day	252.8	167–580
Uric acid, mmol/day	1.85	1.5–4.5
Sodium, mmol/day	145.0	120–220
Potassium, mmol/day	50.5	35–80
Calcium, mmol/day	4.2	2.4–7.2
Inorganic phosphate, mmol/day	24.5	16–64
Chloride, mmol/day	127.5	110–270
Albumin, mg/l	5.25	0–10
Albumin/creatinine ratio	1.5	0–3.5

decay the mutation would lead to the production of a truncated protein product with impaired function lacking most of the extracellular loop between membrane domain 9 and 10, five membrane domains (10–14) and the C-terminal cytosolic domain [18]. Four *SLC4A11* mutations resulting in the introduction of a premature stop codon have been previously detected in probands with Harboyan syndrome (table 2). Presumed pathogenicity of the variant identified here is further supported by its absence in variation databases.

Fig. 2. Specular microscopy imaging in a heterozygous carrier for p.(Arg730*) in *SLC4A11*. Central cornea of the right eye (**a**) and left eye (**b**) compared to a cornea of an age- and gender-matched individual (**c**). Single layers of relatively uniformly sized cells with little variation are shown in all three images (**a–c**).

Table 2. Mutations within *SLC4A11* identified in individuals with Harboyan syndrome

Zygosity	Nucleotide change	Protein change	Location within <i>SLC4A11</i>	Number of families and their origin	Reference
Hom	c.353_356del	p.(Lys118Thrfs*12)	exon 4	1 Indian	19
Hom	c.473_480del	p.(Arg158Glnfs*4)	exon 4	1 Gipsy (Eastern European)	2
Comp het	c.637T>C	p.(Ser213Pro)	exon 5	1 Sephardi Jewish	2
	c.2566A>G	p.(Met856Val)	exon 18		
Hom	c.1378_1381delinsA	p.(Tyr460_Ala461delinsThr)	exon 11	1 Dominican Republican	2
Hom	c.1463G>A	p.(Arg488Lys)	exon 11	1 Moroccan	2
Hom	c.2067-6_16delinsGCCGGCCGG	inactivation of splice acceptor site	intron 15	1 Indian	19
Hom	c.2188C>T	p.(Arg730*)	exon 16	1 Czech	current study
Comp het	c.2233_2240dup	p.(Ile748Metfs*5)	exon 16	1 South American Indian	2
	c.2528T>C	p.(Leu843Pro)	exon 18		
Hom	c.2264G>A	p.(Arg755Gln)	exon 17	2 Indian	19
Comp het	c.2423_2454del	p.(Leu808Profs*60)	exon 17	1 Dutch	2
	c.2528T>C	p.(Leu843Pro)	exon 18		

hom = Homozygous; comp het = compound heterozygous. All variants are annotated in accordance with NCBI transcript NM_032034.3 and their description follows recommendations of the Human Genome Variation Society (www.hgvs.org/mutnomen/). None of the sequence variants listed below was found in the Exome Variant Server for the NHLBI Exome Sequencing Project (evs.gs.washington.edu/) or the 1000 Genomes data set (<http://browser.1000genomes.org/>).

The observation of a rare mutation in a homozygous state may be due to enrichment in a given geographical area, or consanguinity in previous generations unknown to the proband. Homozygous mutations in *SLC4A11* as a cause of CHED2 and/or Harboyan syndrome in the absence of consanguinity have been reported previously [19, 20]. As we were not able to examine other relatives except for the proband's daughter, we cannot exclude the possibility of a larger deletion on the second allele, how-

ever, this type of mutation has not been reported for *SLC4A11* [21].

To the best of our knowledge, we have performed the first detailed assessment of renal function in an *SLC4A11* mutation-positive Harboyan syndrome patient. Two different *Slc4a11*^{-/-} mouse models are described, one presented with decreased urine osmolarity, polyuria and increased total excretion of electrolytes [10], and another with decreased urine osmolarity and decreased concen-

trations of all electrolytes (daily urine output was not possible to be determined) [11], but no kidney abnormalities were identified in the proband described here. Urine osmolality, volume and electrolyte concentrations were all within the normal range.

Therefore we conclude that renal function abnormalities do not seem to be part of the Harboyan syndrome phenotype in humans. However, as the disease shows inter- and intrafamilial variability [19], renal function of other affected patients should be assessed. It is currently not clear why the kidney is affected in the mouse model, but the patient has normal kidney function. It is possible, but unlikely in this case, that some residual SLC4A11 activity remains and is sufficient for renal function, or that there are as yet undetermined tissue-specific alternative isoforms of the gene or the gene has redundant function in human kidney.

The relatively late onset of subjective hearing impairment in our patient supports the previous suggestion that the association of CHED2 and sensorineural deafness may remain undetected especially in younger patients [22]. It cannot be excluded that some patients diagnosed with CHED2 may be found to suffer from hearing impairment later in life, which might improve understanding regarding the genotype-phenotype correlation and increase the number of pathogenic sequence variants associated with this condition.

Specular microscopy examination of the proband's daughter, a heterozygous carrier for c.2188C>T in

SLC4A11, showed no readily detectable abnormalities, supporting previous observations in heterozygous parents of CHED2 patients [5].

At the time the proband was born, very little was known about congenital endothelial dystrophies, thus leading to her initial incorrect diagnosis of congenital glaucoma. Clinical data resulting from a long-term ophthalmological follow-up of our patient may be useful for disease management and counselling of other individuals with this rare syndrome.

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Mutation screening was performed in the DNA Sequencing Laboratory, Faculty of Science, Charles University in Prague. The authors would like to thank Dr. Stepanka Hrda for technical assistance.

Disclosure Statement

The authors declare no conflict of interest.

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